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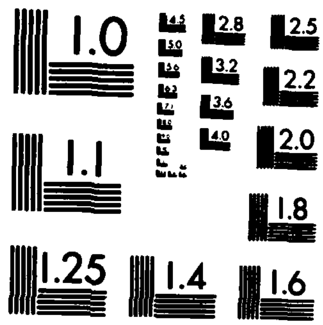

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Seasonal population dynamics, Culicoides spp., treated screens, larvicides, repellents, adulticides

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Population dynamics and control studies on *Culicoides* biting midges were conducted at Parris Island, South Carolina, and Yankeetown, Florida, from September 15, 1979, through September 30, 1983.

Population dynamics included studies on species composition, seasonal incidence, and relative abundance of adult *Culicoides*, and spatial and temporal patterns of larval abundance. Three species at each study site were abundant enough to be considered major pests: *Culicoides furens*, *C. hollensis* and *C. melleus* at Parris Island; and *C. barbosai*, *C. furens* and *C. mississippiensis* at

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Yankeetown. *Culicoides furens* is the dominant species from late spring through early fall. *Culicoides hollensis* and *C. mississippiensis* are predominant in the spring and fall. Plant cover was a good indicator of larval spatial and temporal distribution. Remote sensing techniques were evaluated and found useful for identification of major marsh plant types both qualitatively and quantitatively.

In the laboratory, *C. mississippiensis* was successfully reared at 20°C from eggs obtained from wild-caught blood-fed females. Reared females were 100% autogenous.

Insecticidal control studies included laboratory evaluations of adulticides and larvicides, residual applications of insecticides on treated screens, and ground and aerial ULV applications. These laboratory evaluations identified several promising aerosol adulticides (e.g., resmethrin, malathion, naled), larvicides (chlorpyrifos, temephos) and window screen treatments (permethrin, NRDC-161, propoxur). Aerial applications of naled (Dibrom-14®, 1 oz/acre) were effective in temporarily reducing natural populations at Parris Island.

Promising personal protection techniques included several candidate repellents, deet and four commercial products (Avon's Skin-So-Soft, Claubo®, Johnson's Baby Oil, mineral oil) as skin applications, deet-treated net jackets, and area treatment with repellent-impregnated netting.

OFFICE OF NAVAL RESEARCH

Contract #N00014-79-F-0070

Task No. NR 133-997

FINAL TECHNICAL REPORT

Evaluation of Insecticides, Repellents, and Other Approaches to the Control of Coastal Sand Flies, *Culicoides* spp.

by

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USDA, Agricultural Research Service
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April 1984

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I. INTRODUCTION

Among the varieties of blood-sucking insects that attack man, none can be more annoying than certain species of *Culicoides* biting midges (sand flies). While *Culicoides* have not been shown to transmit disease to humans in the United States, they have been associated with diseases of man in the Caribbean, South America, and other areas of the world (recently reviewed by Linley et al. 1983). Wherever they occur, their bites are extremely annoying, often result in enduring discomfort, and may cause secondary infection (e.g., cellulitis). Because of the location of many military installations, particularly Navy and Marine bases, on coastal tidal areas in the continental United States and the Caribbean, and the widespread distribution of *Culicoides* in these areas, the biting midges can adversely affect the efficiency of personnel stationed at these bases.

In 1976 a survey was conducted of cellulitis cases at the U.S. Naval Hospital, Beaufort, South Carolina. Of 603 patients admitted into the hospital with cellulitis on the upper and lower extremities, it was estimated, based on case histories, that ca. one-third of these cases could be directly attributed to insect bites. The total cases of cellulitis caused an average hospitalization period of 6 days and an annual loss of 3,786 man-days. Examination of the outpatient records from the hospital and the hospital field stations revealed an additional 903 cases of cellulitis. Thus, there were 1,506 cases of cellulitis in 1976, 40% of which required hospitalization and 60% of which required outpatient care. Approximately 500 cases could be directly attributed to insect bites, primarily from *Culicoides*.

A request for assistance to help alleviate this problem was made by the U.S. Navy Hospital Preventive Medicine Officer (J. R. McCormick) to the ARS Insects Affecting Man and Animals Research Laboratory through the Office of Naval Research. Because the necessary data were not readily available to make recommendations on either control or personal protection, this research project was initiated in FY80 to derive the necessary data. The objectives were:

A. To refine existing insecticidal control measures, particularly those that involve chemicals that have been approved for use against mosquitoes by agencies concerned with the protection of man and his environment. These studies were to involve:

   1. Personal protection methods by use of repellents that can assure protection from these pests for extended periods.

   2. Laboratory and field evaluations of both residual and non-residual applications of adulticides to develop immediate remedial control measures. This included studies on timing, formulation, dosage rate, and droplet size of selected insecticides by ground and aerial application equipment.

B. To conduct studies on the population dynamics of coastal *Culicoides*, specifically on species composition, seasonal activity patterns (incidence and relative abundance of both adults and larvae), and diurnal activity patterns of adults, and to further characterize the larval habitat.

This report summarizes the results of four years (FY80 through FY83) of research on these topics, primarily at two study sites, the Marine Corps Recruit Depot, Parris Island, SC, and Yankeetown, FL. Field testing of repellents was also conducted at Fort Myers, FL; Roosevelt Roads, Puerto Rico; and in Panama. Recommendations are made based on the research data accumulated.

II. SUMMARY OF RESEARCH RESULTS

A. Personal Protection and Chemical Insecticidal Control Measures

1. Personal Protection

Four separate personal protection studies were conducted: promising repellent compounds including deet; four commercial products often used and claimed to be effective deterrents against biting midge activity by various members of the public sector; deet-treated net jackets; and area treatment tests with repellent-impregnated netting. Five technical publications resulted from these studies: Harlan et al. 1983, Schreck and Kline 1981, Schreck and Kline 1983, and Schreck et al. 1979a α b. For specific details on techniques the reader is referred to these technical publications or our previous annual reports. Highlights of these studies are included below.

A study was initiated prior to the commencement of this contract to identify promising repellents against natural populations of biting midges at Parris Island (*C. hollensis*) and Yankeetown (*C. mississippiensis*). Five candidate compounds (AI3-30180, 35765, 35770, 36326, and 36536) that had shown good activity in earlier tests against stable flies, mosquitoes and black flies were selected for testing. The candidate repellents were synthesized and purified by standard procedures and supplied by the ARS Organic Chemicals Synthesis Laboratory, Beltsville, Maryland.

All the compounds tested were quite effective; however, AI3-35765 and 36326 appeared to be better repellents against both biting midge species in these studies. These compounds were also outstanding repellents against other biting fly species. AI3-35770 was the most effective material tested, but unfortunately was evaluated only against *C. mississippiensis*. With the exception of AI3-36536, more bites were recorded on the deet standard than on all other treatments at both sites and with both species. In terms of the ratios of mean number of bites, AI3-35770, 36326, and 35765 were 6 to more than 15 times more effective than deet against *C. mississippiensis* and AI3-36326 and 35765 were 3 to 5 times more effective than deet against *C. hollensis*. Though not as effective as the other compounds in these studies, deet did give good protection in most of the tests and should be considered an effective repellent against these species when used as a 25% formulation properly applied to exposed skin. In all tests the untreated control subject averaged 28 times more *C. mississippiensis* bites than the deet standard and 190 times more than the average number of bites from all other materials. Similarly, against *C. hollensis* the check averaged 48 times more bites than deet and 138 times more than the experimental compounds. Avon's Skin-So-Soft (SSS), Johnson's Baby Oil (JBO), Claubo_, and mineral oil were field-tested as skin applications against 6 species (*C. barbosai* Wirth and Blanton, *C. floridensis* Beck, *C. furens* Poey, *C. hollensis* [Melander and Brues], *C. mississippiensis* Hoffman, and *C. melleus* [Coquillett]). These products were applied at full strength. A deet standard was applied as a 12.5 or 25% ethanol solution. At Yankeetown against a mixed population (58% *C. mississippiensis*, 17% *C. barbosai*, 13% *C. floridensis*, and 12% *C. furens*), no bites were recorded on the 25% deet treatments, and biting rates on the candidate treatments were quite low (0-<4 bites/hr) except for Claubo at ca. 23 bites/hr. The untreated check received 384 bites/hr. At Fort Myers, FL, against a mixed population (94% *C. barbosai*, 5% *C. furens*, and 1% *C. floridensis*), SSS was ca. 6 times and Claubo 3 times more effective than deet. JBO was least effective (ca. 120 bites/hr) when paired with deet (ca. 8 bites/hr). However, when these results are compared with the untreated check (ca. 2,511 bites/hr on 1 arm), it must be concluded that considerable protection was afforded by all treatments in these tests. At Parris Island, SC, against a mixed population containing 80% *C. hollensis* and 20% *C. melleus*, <1 bite/hr was recorded for the JBO treatment, and no bites were recorded with the other candidates. The deet standard averaged ca. 2 bites/hr during the same period, while the check averaged 351 bites/hr. These data indicate that each of these commercial products might serve as a useful substitute in the absence of a suitable conventional repellent. However, we observed that protection was achieved primarily because of the oily coating, which trapped the midges on the skin surface, rather than because of complete repellency. One of the materials, Skin-So-Soft, is marketed as a bath oil to be used at the rate of 1/2 capful in a bathtub of water; since it is not labeled for application at full strength, further investigations may be warranted to determine the effect of repeated long term skin applications.

Prior to initiation of the present contract, we (Schreck et al. 1979a) had field-tested the effectiveness of deet-treated net jackets against 4 species of biting midges (*C.*

barbosai, *C. furens*, *C. hollensis* and *C. mississippiensis*) at Parris Island, Fort Myers, Yankeetown, and Roosevelt Roads (Puerto Rico). We found that the jackets provided ca. 98% protection against 3 of these species, but only 59% against *C. barbosai*. During this contract period additional tests were conducted in Panama against a natural population of mixed composition (*C. alahialinus*, *C. barbosai*, *C. furens*, *C. guyanensis* and *C. gorgasi*), which changed from predominantly *C. furens* (94%) to predominantly *C. barbosai* (96%) during the study. In the Panama tests the deet-treated jacket provided 87-93% protection. Persons wearing the deet-treated jackets received from 2-258 bites/hr, whereas unprotected persons received from 60-2507 bites/hr, a difference of 10 to 30 times more bites. Thus, protection observed was not as good against *C. furens*, nor was it as poor against *C. barbosai* as had been reported in our earlier study. These jackets are presently stocked in the U.S. Navy, Marine Corps and Air Force supply systems and available in the retail market for the general public.

These results with deet-treated net jackets indicated the potential spatial action of deet. On the basis of these studies, we initiated a preliminary field trial in which a 3 X 3 m area was protected from intrusion by *C. mississippiensis* by a 2 m high netting of deet-treated, lightweight cotton/polyester netting around the perimeter. Enclosures 2 m high and up to 144 m^2 were used in subsequent tests. The perimeter netting was treated with deet, diisopentyl malate (DPM) or indalone to determine if the biting midges (*C. barbosai*, *C. floridensis*, *C. furens* and *C. mississippiensis*) attracted, by CO_2 metered from a cylinder or by human subjects within the enclosures, could be prevented from entering the area. Deet and DPM were tested at 0.125 and 0.25 g AI per g of netting (200 cm^2) and indalone only at the higher rate. During the initial tests an untreated enclosure adjacent to the treated ones was used as a comparison. Thereafter, only biting rates inside and outside treated enclosures were compared. Greater numbers of midges were always (13 tests) recorded from the untreated enclosure (1239.6±2094.7 SD) than from the "outside" trap (627±1341.3 SD), indicating that the untreated net did not provide a physical barrier to biting midge intrusion; also, the standard deviations indicate the variability in midge density from day to day. Based on CO_2-baited sticky trap data, in 24 hr the 0.25 g deet treatment provided the 72 m^3 space with over 99% protection (ca. 0.05 midges/hr), while a 288 m^3 space was provided with 92% protection (ca. 13 midges/hr). In tests with human subjects, 99% protection was provided after 24 hr for a 288 m^3 space treated with deet at the higher rate. The residual effectiveness for 8 tests was >89% protection for up to 96 hr after treatment when no rain fell on the netting. When rainfall occurred the repellency of the deet treatment was quickly lost.

The 0.125 g DPM treatment provided a mean of 96% protection 24 hr after treatment (288 m^3 enclosure). At 120 and 144 hrs (inclement weather prevented making 48 and 96 hr evaluations) this treatment gave only ca. 53% protection. Treatments of 0.25 g provided >86% protection up to 5 days (120 hr) after treatment.

Indalone (0.25 g and 288 m^3 enclosure) was erratic in its effectiveness. Protection at 24 hr after treatment was only 46%, with relatively low numbers of biting midges present. At 48 hr, however, with relatively high numbers of midges, 87% protection was achieved. At 72 hr, protection had dropped to 69% and by 96 hr to 60%.

2. Chemical Insecticidal Evaluations

Chemical control studies with candidate insecticides can be divided into 5 main areas: laboratory screening of aerosol adulticides, droplet size evaluation, residual applications on household screens, larvicides, and field testing of ground or aerial ULV applications.

a. Relative toxicity tests (wind tunnel evaluations of adulticides).

We felt that compounds registered for control of mosquitoes, if used properly, can give some temporary local reductions in biting midges. Therefore, the assessment of insecticidal aerosols against adult *Culicoides* was an essential part of our research program. The wind tunnel system used in our evaluations was basically that described by Mount et al. (1976), but several changes were necessitated by the small size of the adult biting midges. The major change was the substitution of the 16-mesh galvanized screen wire with 40-mesh brass screen for the exposure cage. Also, the fabricated atomizing nozzle was changed to a commercially available standard stock nozzle (#12891 1/8JJ, Spraying Systems Co., Chicago, IL). The wind tunnel consists of a cylindrical tube 15.5 cm in diameter through which a column of air is blown at a rate of 6.4 km/ha. Approximately 25 adult female biting midges are confined in cardboard exposure cages, 8.6 cm in diameter and 5.0 cm high, with 40-mesh brass screen ends. The cages are placed in the center of the tube for exposure. Using an automatic pipette, 0.25 ml solution of the desired concentration of the technical insecticide in acetone (wt. AI/volume diluent and expressed as % concentration) is atomized at 105.5 g/cm^2 into the upwind portion of the tube and the insects are exposed momentarily as the aerosol passes through the cage. Following exposure, the insects are lightly anesthetized with carbon dioxide and transferred to new 8.6 X 5.0 cm cardboard holding cages covered with fine mesh cloth screen tops. Cotton pads soaked in a 10% sucrose solution are placed on the cloth screens to sustain the insects until all mortality counts are made. The holding cages are placed in large styrofoam chests containing moistened cotton pads to maintain a high humidity environment. Knockdown is checked 1 hr after treatment, and mortality 24 hr after treatment. Checks are exposed to contact sprays containing acetone only and handled in the same manner. This test is not intended to yield results that can be compared closely; however, it does eliminate those insecticides that are not toxic enough to warrant further testing, and it identifies the range of discriminating concentrations for those insecticides that are highly effective.

In the first of 2 sets of wind tunnel tests that were conducted, the effectiveness of 4 synthetic pyrethroids (NRDC-161, permethrin, resmethrin and d-phenothrin) and 3 organophosphorus (OP) compounds (fenthion, malathion and naled) was tested against field-collected adult female *C. mississippiensis*. The pyrethroids were more effective than the OP compounds tested. NRDC-161 was the most effective pyrethroid, followed by permethrin, resmethrin and d-phenothrin. Of the OP compounds, naled was slightly more effective than malathion, and fenthion was the least effective. These data have been published (Kline et al. 1981).

In the second study 4 OP compounds, malathion, chlorpyrifos, sumithion and Montedison M 9580 (AI3-29473) and the carbamate propoxur were tested. The only compound common to both studies was malathion and the LC-50's obtained for this chemical in both studies were almost identical, 0.0239 vs. 0.0233% (wt. AI/volume diluent, expressed as % concentration). Based on LC-50's, chlorpyrifos was the most effective of these compounds, followed in decreasing order by propoxur, malathion, sumithion and AI3-29473. Chlorpyrifos was slightly more effective than naled, which was the most effective OP compound tested in the first study. Propoxur was slightly less effective than naled.

b. Droplet-size Studies

In mosquito control, aerosol droplet size influences insecticidal efficiency (Mount et al. 1975). To determine the effect of droplet size on insecticidal efficiency on biting midges, laboratory wind tunnel tests were conducted with caged adult female *C. mississippiensis*. A Berglund-Liu Monodisperse Aerosol Generator (Thermo Systems, Inc.) was used to introduce uniform size droplets into a wind tunnel for exposure of insect samples. Droplet size was varied by a technique involving evaporation of a volatile solvent (isopropanol) from a solution containing a nonvolatile insecticide (malathion) at different concentrations. Exposure time was varied to provide different dose levels. A series of tests with field-collected *C. mississippiensis* adult females was conducted for 3 aerosol sizes (2.8, 4.8 and 8.2 μm diameter) at 6 dose levels. Mortality readings were made at 1, 2, and 24 hr after treatment and each treatment was replicated 4 times on different days. The results showed an increase in mortality (lower LD-50) with time after treatment due to a slow response to malathion. The smallest aerosol size (2.8 μm) was clearly less efficient than the 2 larger sizes (95% fiducial limits did not overlap at 2 and 24 hr posttreatment). The largest size (8.2 μm) produced the lowest LD-50 at each time; however, the difference in effect between 4.8 and 8.2 μm droplets was not large (95% fiducial limits overlapped at each reading). The leveling-off tendency between 4.8 and 8.2 μm indicates that the optimum size is not much, if any, greater than 8.2 μm. These results indicate that aerosols from standard ground ULV generators with a volume median diameter (VMD) of 8-10 μm should be highly efficient for biting midge control if applied in a manner that provides adequate exposure of the target insects.

c. Evaluation of treated window screens

Several studies were conducted on the effectiveness of residual applications of candidate insecticides on household screens (16 X 18 mesh) against natural populations of *C. mississippiensis*. The tests were conducted in divided test chambers consisting of 2 cylindrical paper cartons (430 cm^3 each(described and illustrated by Jamnback (1961, 1963) and Dukes and Axtell (1976). The insecticides were formulated as acetone solutions with technical grade material at concentrations expressed as percent AI (wt./vol.) and agitated thoroughly just before use. Discs (9 cm diameter) of screen were treated by immersion for ca. 10 sec in the solution. After treatment, the screens were hung beneath the eaves of a building to dry. Control tests were conducted with screens immersed in acetone only, The effectiveness of the screens was tested ca. every 7 days posttreatment, depending on the availability of indigenous biting midge adults. After each test, the screens were removed from the test chamber and hung outside under the eaves of a building to age and weather. Criteria for effectiveness were knockdown capability (1 hr mortality), high toxicity (4 hr mortality), and longevity of the insecticide when weathered.

In the first study, chlorpyrifos, fenthion, malathion, and propoxur were chosen in concentrations of 1, 3, 5, and 8% for evaluation on aluminum screens. Propoxur at 8% was the most effective with a range of 73 to 92% knockdown over 35 days and 97 to 100% overall mortality. Chlorpyrifos (8%) gave 97 to 100% overall mortality for 35 days but was inadequate in terms of quick knockdown. Malathion (8%) was effective only 14 days, and fenthion was ineffective at any of the concentrations tested. These results suggested that screen treatments at 21-day intervals with 8% propoxur may provide significant relief.

Additional screen studies were conducted with 2 synthetic pyrethroids, permethrin and NRDC-161 (Decamethrin®). Permethrin was tested at 0.125, 0.25, 0.5 and 0.75% AI, and NRDC-161 at 0.125, 0.25, and 0.5% AI (wt./vol. technical in acetone). NRDC-161 was the more effective compound with ca. 100% mortality (at 1 and 4 hr) against biting midges exposed to the 0.25 and 0.5% treated screens through 168 days posttreatment. After 168 days, we had difficulty trapping midges for testing. The 0.125% treated screens caused >90% mortality through 35 days. Screens treated with permethrin caused >90% mortality for 95 and 35 days posttreatment at the 0.75 and 0.5% concentrations, respectively. Knockdown (1 hr) was >90% for both concentrations for 35 days after treatment, and then dropped off sharply (<50% by 42 days). Greater than 90% mortality was achieved for only 7 days posttreatment on screens treated with 0.25% permethrin, and screens treated with 0.125% permethrin gave <50% mortality 1 day after treatment. Based on these data, a single treatment of screens with permethrin (0.75% or NRDC-161 (0.25%) should give excellent protection for an entire *C. mississippiensis* season. These data have been included in three technical publications (Kline and Roberts 1981, Roberts and Kline 1980, 1981).

d. Evaluation of candidate larvicides

Larvicide tests were designed to determine the toxicity of acetone-water suspensions of candidate insecticides to 3rd and 4th instar larvae. Four organophosphorous insecticides, chlorpyrifos, fenthion, malathion, and temephos, were tested. Specific objectives were to determine the relative toxicities of these 4 compounds, and the effect of substrate soil on effectiveness (Kline et al., in review). Since colonized insects were not available, larvae were extracted from the salt marsh substrate for testing at several discriminating concentrations (ppm). Tests were run in duplicate, exposing 20 larvae to each concentration level, and replicated 3 times. Since the insecticides were formulated in acetone, duplicate beakers of acetone-water solutions were set up as controls.

The relative potency of the 4 chemicals varied greatly, with chlorpyrifos the most, and malathion the least toxic. Against these field-collected larvae, 9 times more temephos, 22 times more fenthion, and 176 times more malathion was required to achieve the 50% mortality level compared to chlorpyrifos in water. Addition of salt marsh substrate did not change the order of effectiveness, but 9 times more chlorpyrifos, 2.3 times more temephos, and 1.3 times more fenthion or malathion than chlorpyrifos was required to cause the same mortality as estuarine water alone. The data indicate that these insecticides, with the possible exception of malathion, merit further testing under field conditions.

e. Field Tests

Ground ULV tests with resmethrin were conducted at Yankeetown against natural *C. mississippiensis* populations. Effectiveness of the sprays was assessed by population monitoring with CDC light traps baited with CO_2, with caged mosquitoes or biting midges, and with cages containing both mosquitoes and biting midges. The combination was necessary in order to test penetration of the spray through the small mesh screen used to confine the biting midges. The resmethrin was sprayed at the rate of 0.007 lb/acre from a vehicle moving at 10 mph and delivered at 4 oz/min in the first test, and at 9.2 oz/min in the second test.

The results were indeterminate. In test 1, high mortality was obtained in all cages at 2 locations, and slight to no mortality at the other locations along the treatment route. In test 2, high mortality in the control cages negated the test cage results and, although there was some indication that the field population was reduced, high variability in collection numbers prevented accurate assessment. One major problem in achieving control appears to be the timing of spray application. *Culicoides mississippiensis* is active for several hours before sunset but only briefly after sunset; therefore, treatments were made ca. 2 hr before sunset. At this time of day, thermal air currents, especially over those sections of the road exposed to the sun, prevented proper aerosol dispersion. A 2nd factor in obtaining adequate coverage with ground ULV equipment is the influence of vegetative cover which, when dense, appears to restrict movement of the aerosol through the area. Based on these limited observations, it is likely that installations in coastal areas will be able to utilize ground ULV applications only sparingly.

Aerial application tests for control of *Culicoides* were conducted at Parris Island in cooperation with the U.S. Air Force's 355th TAS/Aerial Spray Team. the purpose of these tests was to evaluate the effectiveness of ULV aerial applications of Dibrom® 14, an 85% formulation of naled, against natural populations of adult biting midges on Parris Island.

The 1st test occurred on April 7, 1981, and consisted of 2 applications of a 1:5 mixture of Dibrom 14: heavy aromatic naphtha (HAN) at a rate of 1.5 oz/acre (0.25 oz/acre Dibrom 14) each spaced ca. 20 min apart to take advantage of any flushing action from the 1st application. This resulted in a total application rate of 1/2 oz/acre of Dibrom 14, which is the minimum dose recommended for adult mosquito control. Two Air Force UC-123-K aircraft applied the insecticide using techniques normally employed for mosquito adulticiding. The entire land and marsh area of Parris Island (ca. 8000 acres) was treated. The aircraft were equipped with Tee Jet nozzles oriented 45° forward and were flown along 2000-ft swaths at 140 kts airspeed and 200 to 250 ft altitude. The effectiveness of the insecticide treatment was determined by measuring the population with surveillance traps and by bioassays with caged insects.

Results of the natural population surveys and caged insect bioassays indicated that only a low level of control resulted from these minimal-dosage aerial applications. The surveillance traps indicated that the population level actually increased in both the treatment and control areas; however, the population level in the treated area was ca. 50% lower than the level in the control area. Since the pretreatment population levels for both areas had been nearly the same this suggests that the insecticide achieved around 50% mortality in the treated area. This agrees with the results of the caged insect bioassays where the overall mortality of caged mosquitoes and biting midges was 67% and 58%, respectively.

A number of factors probably contributed to the low level of control. For example, the likelihood that there was insufficient penetration of the spray in vegetated areas was indicated by the 87% mortality of caged mosquitoes placed in open or sparsely vegetated areas vs. 40% in cages protected by dense vegetation. Although the number of caged biting midges was limited, their mortality was also greater in open areas. The low dose applied in this test may have been insufficient to provide the penetration expected at normal dosage levels. Other environmental factors such as marginally high winds and low temperatures during the application could also have adversely affected the treatment.

In 1982 (April 21-22), applications of undiluted Dibrom 14 at the rate of 1.0 oz/acre each were applied on 2 consecutive days over the entire island. These applications were timed to coincide with the first spring population peak of biting midges and suitable weather for aerial applications. This time the application was made by a single UC-123-K from an altitude of 150-250 ft using 1000-ft swaths. Each application was made in the late afternoon starting ca. 1800 EST and finishing before dark.

The effectiveness of the applications was again measured by a combination of natural population survey and caged insect (when available) bioassay. Caged insects were not available during the 1st application, but both caged mosquitoes and biting midges were used during the 2nd application. A limited number (4-6) of cages of both insects were also placed in the check areas to monitor natural mortality in the cages.

This time the survey trap collections indicated a very significant reduction in the natural population of both *Culicoides* spp. and mosquitoes. Trap collections in the check area, which were consistently lower than those on Parris Island, remained stable throughout the test period. The reduction in the numbers collected in traps in the treated area on the day following the 1st application (before the 2nd spray) was only 63%. However, this value reflects the collection that occurred during and immediately after the spray application as well as insects that subsequently were attracted to the traps. Thus, the data for the 1st captures do not fully reflect the impact of the 1st spray. This is particularly true for *Culicoides* spp., which showed a temporary period (ca. 5 min) of increased biting activity immediately after the spray plane passed overhead. The overall impact of both sprays was clearly shown by a reduction of greater than 99% in trap collections on the day following the 2nd application. This level of suppression was maintained for more than 3 days following the last treatment.

Further evidence of the effectiveness of the sprays was indicated by the high mortality of caged insects in the treated area, which averaged 96% for mosquitoes and 95% for *Culicoides* spp. Only insects in cages placed at the northern (windward) end of the spray area had significantly less than 100% mortality. The upwind swath offset was probably insufficient to give good spray coverage in this area. However, this was not detrimental to the overall effectiveness of the application. The kill of the caged insects combined with the natural population suppression indicated that good penetration of the vegetation was obtained with the higher dose applied in this test (1 oz/acre/treatment).

These 2 sprays showed that conventional aerial applications of insecticides can be effectively used to control *Culicoides* spp. in non-isolated coastal areas when the applications are made with sufficient dose levels. These data have been published (Haile et al. 1984, in press).

Based on these results, in 1983 three aerial sprays were conducted at 2 week intervals in the spring, followed by 1 fall application. For these tests we used undiluted Dibrom 14 at a rate of 1 oz/acre. Bioassays, consisting of caged mosquitoes and biting midges, and CO_2-baited CDC traps to monitor the natural populations were used to assess the effectiveness of these sprays. Mortality of the caged mosquitoes and biting midges was ca. 99% for each spray. Analyses of the surveillance trap collections of the day after each treatment showed that the natural populations of the biting midges were reduced by 95, 80, 86, and 98%, respectively, for the 4 sprays, compared to the day prior to treatment. Weather conditions were ideal during the 1st and 4th tests; winds were marginally high during the 2nd test, and were almost too calm at spray time on the 3rd spray. For at least 3 days after each spray very few complaints of biting activity were reported. During these same periods base personnel with homes in nearby untreated communities reported that biting midge activity was intense.

While these results represent considerable progress, we feel that additional field studies are needed to further define the effect of chemical dilution and application interval on overall efficiency and duration of control. Further field studies will also provide additional information concerning optimum weather conditions for aer[illegible] applications of insecticides.

B. Population Dyn[illegible]

Field and [illegible] studies were conducted to gain insight into behavioral, [illegible]al and ecological aspects of salt marsh species of *Culicoides* [illegible]at might lead to improved insecticidal application measures. This information might also be useful in developing new and more effective control strategies. Most of these data are now being closely scrutinized and are undergoing detailed analysis. Few of these data have been published to date, but most of the studies are in various stages of preparation for submission as technical publications. A list of proposed titles can be found on pages 24-25.

1. Adult Species Composition and Seasonal Abundance

New Jersey (NJ) light traps are the most commonly used method for determining species composition and seasonal incidence of adult *Culicoides* spp. in a general geographical area. Trap collections made over an extended period give an indication of what species are most common, and in which months the various species are most abundant. Throughout the duration of this contract, adult species composition and seasonal patterns have been monitored with modified NJ light traps at Parris Island and Yankeetown. Each NJ trap was equipped with a 40-watt incandescent light bulb, an automatic timer, and 40-mesh brass screening in lieu of the regular screen of the delivery cone.

At Parris Island, four NJ light traps were operated nightly. Samples were retrieved from the field at least twice weekly (3- and 4-day intervals). Three species, *C. furens* Poey, *C. hollensis* Melander and Brues, and *C. melleus* (Coquillett) were abundant enough to be considered as major pests. The thirteen other *Culicoides* species collected were *arboricola* Root and Hoffman, *baueri* Hoffman, *biguttatus* (Coquillett), *crepuscularis* Malloch, *guttipennis* (Coquillett), *haematopotus* Malloch, *hinmani* Khalaf, *nanus* Root and Hoffman, *niger* Root and Hoffman, *piliferus* Root and Hoffman, *stellifer* (Coquillett), *venustus* Hoffman, and *villosipennis* Root and Hoffman; these comprised <1% of the total trap collections.

Seasonal patterns were very similar for all 4 years of this study. *Culicoides furens* was present in trap collections from mid-April through late October with definite peaks in May, June, August, and late September-early October. *Culicoides hollensis* was most abundant during October through mid-November. Only an occasional specimen was collected from mid-November through February. Generally, *C. melleus* peaks several times during early spring. In 1982 it peaked in early spring but remained at a high level throughout the summer months, and then reached a fall peak from October through mid-November.

At Yankeetown three modified NJ light traps were operated nightly. Collections were retrieved daily except for weekends. Three species, *C. barbosai* Wirth and Blanton, *C. furens* and *C. mississippiensis*, were the predominant species. The 17 other *Culicoides* species collected are listed in order of decreasing abundance: *arboricola, floridensis* Beck, *haematopotus, melleus*, n. sp. 109 near *baueri, ousairani* Khalaf, *insignis* Lutz, *bermudensis* Williams, *stellifer, loughmani* Edwards, *hinmani, crepuscularis, paraensis* (Goeldi), n. sp. near *spinosus, biguttatus, niger*, and *nanus*; together they comprised <1% of the total number of specimens collected. *Culicoides mississippiensis* was present throughout the year, but was abundant only in the spring and fall. The population began to increase in February and peaked in May-early June. It often persisted throughout the summer and early fall in very low numbers, followed by a sharp rise in abundance during November and early December. From mid-December through mid-February it occurred in low numbers. *Culicoides furens* first appeared in early April, reached several definite peaks from May through August, and disappeared from the collections in late October-early November. *Culicoides barbosai*'s incidence paralleled that of *C. furens* but at a much lower level of abundance.

2. Diurnal Flight Activity

Either Koch-type (Koch et al. 1977) or modified Koch-type suction traps were used to study diel flight activity patterns of adult *Culicoides*. Each suction trap divided the catches into 12 one-hr samples using a turntable collector. Two suction traps were operated at each study location, one each for consecutive 12-hr periods so that the total day's activity was monitored on an hourly basis.

At Parris Island, 2 Koch-type suction traps were operated 5 days per week for 1 year (March 1980 to March 1981). Three major species of *Culicoides* were captured in these traps and were dominant at different periods in the spring: *C. hollensis* from March to mid-April, *C. melleus* from mid-April to mid-May, and *C. furens* from mid-May to June. Spring peaks of diurnal activity varied: *C. hollensis*, 7-9 AM (EST) and 6-3 PM; *C. melleus*, 5-7 AM and 7-9 PM; and *C. furens*, 5-6 AM and 6-7 PM. Numerous males of all 3 species were collected. No *C. hollensis* were collected from mid-June until September. Very few *C. melleus* were caught in these traps during summer and fall, even though they were abundant in the NJ light traps. *Culicoides furens* was abundant in these traps throughout the summer and early fall with peak activity periods between 5-8 AM and 8-10 PM. *Culicoides hollensis* was abundant in October and November with peak activity between 7-9 AM and 4-7 PM.

At Yankeetown 2 modified Koch-type interval suction traps were operated ca. 4 days each week from September 1981 through April 1983 except during January and February 1982. The traps were modified to include a NJ type trap with a 40-watt incandescent light source and were baited near the light source with CO_2 (flow rate of 500 ml/min) from a pressurized tank. Separate 24-hr timers were connected such that each trap collected for alternate 12-hr periods. The data show that *C. mississippiensis* is often active throughout the day but with a definite preference for the late afternoon. This species' greatest flight activity was between 4-7 PM EST in the fall (largest peak 5-6 PM) and 4-11 PM in the spring (largest peak 6-7 PM). *Culicoides furens* was most abundant in collections made in October and from May through September. This species was most active between 8 PM and 6 AM (largest peak was 9-10 PM). *Culicoides barbosai*'s seasonal patterns were very similar to those of *C. furens* and the maximum flight activity occurred between 6-10 PM.

3. Immature Habitat Characterization and Population Dynamics

Substrate sampling was utilized to determine the presence, spatial and seasonal distribution, and relative abundance of immature stages of salt marsh *Culicoides* spp. At Parris Island, due to limited manpower, comparatively little time was spent on substrate sampling until October 1982. Then, the initial emphasis was to locate and characterize all potential breeding sites. Twenty-one different types of potential habitats were identified. Generally, extensive areas of open *Spartina* marsh yielded very few larvae (ca. 5 larvae/sample), but 2 habitats that consistently yielded larvae were the upland edge of the marsh (ca. 30 larvae/sample) in those areas that were shaded by live oak trees, and under the trunks of trees which had fallen into the *Spartina* marsh (ca. 25 larvae/sample). Areas covered with sparse *Salicornia* or completely denuded panne areas and mudflats produced very few larvae (<1 larvae/sample). We also noted that the more uniform an area in terms of vegetation, substrate, moisture gradient, and distance from a tidal creek or depression, the more uniform was the resulting density. Also, on a seasonal basis, more larvae/sample were generally obtained during the months January through March; the lowest numbers of larvae/sample were obtained during April through June. This was not unexpected since this pattern is the inverse of adult abundance.

At Yankeetown much effort was devoted to habitat characterization and determination of spatial and seasonal distribution of immature *Culicoides*. Studies conducted prior to initiation of this contract had shown that *C. furens* and *C. mississippiensis* utilize the intertidal plant communities as breeding locations. Such information is essential before control measures can be directed against immature stages. We felt that if larval distribution could be more definitely delineated, spatially and temporally, then larvicide treatments might be restricted to the productive areas. In 1980 our main study area was established within a 125 X 425 m section of typical salt marsh, subdivided into 85 25 m^2 plots. Each plot was characterized according to major vegetative cover type, *Spartina, Juncus, Distichlis*, and various mixtures of these grasses. Weekly substrate samples were taken from each of the 3 major vegetative types. The location of each sample was determined through random selection of the plots in which the desired vegetation type was found. Larvae were recovered by the agar extraction technique (Kline et al. 1981). A portion of larvae recovered from each vegetative type was reared for sex and species determination. This study was conducted for 19 consecutive months, during which 74 sampling trips were made and 2207 usable substrate samples were collected. Eighty-one percent of the samples yielded at least 1 *Culicoides* larva. A total of 19,923 larvae were recovered, an average of 8.9 larvae/sample. When relative larval production figures are based on the 3 major vegetative habitats, *Distichlis* areas produced a significantly greater overall mean per sample (11.2 larvae) and accounted for the greatest relative percentage (41.8%) of the total larvae recovered during the entire sampling period. The combined *Spartina* areas produced an intermediate mean of 8.8 larvae per sample (32.8%). *Juncus* areas produced a mean of 6.8 larvae per sample (25.4%). Approximately 25% of the samples taken from both *Juncus* and *Spartina* areas yielded no *Culicoides* larvae, while only 12% of the samples taken from *Distichlis* areas showed negative results. Although these data provided a measure of larval density for each vegetation type, extrapolation of the data to take into account the total area of each type revealed a different view of the overall contribution of *Distichlis* (11.2% of the larvae), *Spartina* (25.2%), and *Juncus* (63.6%).

In terms of seasonal occurrence, the mean number of larvae recovered per sample from all habitat types combined showed a general increasing trend from May 1980 to June 1981, ranging from a low of 4.8 (August 1980) to 18.6 (June 1981). Overall populations after June 1981 declined and then stabilized at 8.3 larvae per sample for the last 3 months of the study (September-November 1981).

Some larvae were found in all vegetation types throughout the year but the relative distribution varies. The overall trend was that more larvae were recovered in *Distichlis* areas during the periods of greatest flooding (May-September in both 1980 and 1981) and in *Spartina* areas during the period (December 1980-April 1981) of least flooding of the marsh.

These data on seasonal trends become more meaningful when the emergence data are considered. Based on total numbers of unidentified larvae, there were only 3 months during the study in which 1 vegetation type produced significantly greater numbers of larvae than all the other vegetative types. *Culicoides furens* and *C. mississippiensis* were the only species which emerged from reared larvae. A significantly larger number of *C. mississippiensis* (6.5/sample) occurred within

Spartina habitats than in *Juncus* areas (4.5/sample) or *Distichlis* habitats (2.0/sample). A significantly greater number of *C. furens* (8.6/sample) occurred within *Distichlis* areas than in *Spartina* and *Juncus* areas (ca. 2.5/sample) each.

The next step in our larval studies was to compare larval populations in the gridded area with those in surrounding marsh areas. For this purpose, transects were established throughout the Yankeetown marshes and larval densities from similar vegetation types on and off the grid were compared during a 10-wk period in the summer (1982) and again in the winter (1983). During this study we noticed that marsh lands bordering the Gulf of Mexico in the vicinity of Yankeetown are stratified with respect to their distance from the Gulf. Aerial photographs and ground truthing revealed that the marsh areas are divided into zones 1 and 2. Zone 2 marshes occur at higher elevations and are characterized by few tidal creeks and subsequently less frequent flooding. The gridded area was typical of zone 1 marshes. Overall density for the summer months (larvae/sample) among the 3 areas was similar: grid (9.9), zone 1 (9.0) and zone 2 (9.2). In the comparison of vegetation associations, *Distichlis* areas were the most productive (11.4) followed closely by *Spartina patens* (11.1), then by *Juncus* (9.6), *Spartina alterniflora* (8.2), and lastly by margins of ponds (4.8) (found only in zone 2). These data were in general agreement with those obtained in our 19-month grid study. During the summer months *Distichlis* areas are more productive than *Spartina alterniflora* areas. We believe that the major reason for this seasonal occurrence is due to tidal dynamics. During the summer months, the tides are consistently higher (ca. 0.3 m above mean low water) than during the winter months. Therefore, *S. alterniflora* is inundated for very extensive periods of time during the summer months.

When the same marsh areas were resampled during the low tide cycle (January through March), overall larval density for the 3 areas was: grid (4.9), zone 1 (7.5) and zone 2 (4.7). All these numbers reflect lower overall densities than those observed in the summer sutdy. In the comparison of vegetation associations, *Spartina alterniflora* areas were the most productive (9.7) followed by *Spartina patens* (6.1), margins of ponds (5.5), *Distichlis* (4.5) and *Juncus* (4.3).

These data indicate that plant cover can be used as an indicator of larval density and that there is a seasonal shift in which plant zone the majority of larvae are found. However, to utilize this type information in a pest management scheme, it would be impractical for ground crews to map the various plant cover types. Therefore, the next step in our study was to expand our study on the feasibility and practicality of using aerial color infrared photography. Remote sensing flights were conducted in cooperation with the USDA

Citrus Insects Research Laboratory, Weslaco, Texas, in May and September 1983. Assessment of the resulting photos indicate that this technique will be very useful in distinguishing pure stands, as well as various mixtures, of the different types of marsh vegetation found in the Yankeetown area. At the conclusion of this contract, therefore, contact has been made with the recently established (late 1983) NASA remote sensing laboratory on the University of Florida campus, Gainesville, FL, to determine the extent to which we could analyze color infrared negatives. It appears that computer enhancement of our negatives will allow us to quickly map out, qualitatively and quantitatively, any marsh areas with similar vegetation characteristics.

4. Laboratory Life History Studies

We studied the fecundity of blood-fed *C. mississippiensis* females and the effect of temperature on development of immature stages with the goal of establishing a laboratory colony. The results of this study have been published (Davis et al. 1983b). The average fecundity of blood-fed females was 35 eggs per female and the mean number of hatched eggs was 10.3 per female. Development of immature stages was studied at 5 constant temperatures (10, 15, 20, 25 and 30°C) and was successful at 15, 20 and 25°C. Development times were inversely related to temperature, with the optimal temperature for rearing being 20°C. Complete development at this temperature required a total of 57 days.

Techniques which have been published (Davis et al. 1983a) were developed for the preparation and use of a reinforced silicone membrane for feeding of *C. mississippiensis* on preserved bovine blood. No significant differences were noted among females fed, respectively, on a human host, defibrinated bovine blood, and citrated bovine blood in the mean number of eggs matured (40±11; 35±12; 37±13) or in the percentage of egg hatch (72±5%; 61±13%; 68±6%). This development eliminates the need for live laboratory animals or human hosts for routine colony maintenance.

III. ACCOMPLISHMENTS

A. Laboratory studies were completed to determine the toxicity of candidate insecticides to 3rd and 4th instar *Culicoides* larvae.

B. Laboratory wind tunnel tests were conducted with candidate insecticides against field-collected adult female *Culicoides mississippiensis*. Twenty-four hr mortality observations revealed the following order of effectiveness: NRDC-161, permethrin, resmethrin, d-phenothrin, naled, chlorpyrifos, propoxur, Montedison AI3-29473, malathion, fenthion, and sumithion.

C. Seven candidate chemicals have been evaluated for their potential use as residual applications on household screens to retard entry of adult *Culicoides* into buildings. Based on knockdown observations of adult *C. mississippiensis*, the most effective insecticide evaluated was NRDC-161, followed by permethrin, propoxur, malathion and chlorpyrifos, all of which far outranked fenthion and an experimental OP compound. Listed in order of decreasing residual toxicity based on 28 days posttreatment were NRDC-161, permethrin, chlorpyrifos, propoxur, malathion, fenthion and the experimental OP compound.

D. In field studies, 5 selected repellents were evaluated in paired tests with a deet standard against *C. hollensis* at Parris Island, SC, *C. mississippiensis* at Yankeetown, FL, and *C. barbosai* at Fort Myers, FL. In terms of numbers of bites received when tested against all 3 species, the most effective compound, AI3-36325, gave 99% protection; the least effective compound, AI3-36346, gave 92% protection while deet gave 95% protection.

E. In similar tests against *C. mississippiensis* and *C. barbosai*, several commercial preparations which had been claimed to be effective against biting midges were compared to deet in standard paired tests. The preparations were Avon's Skin-So-Soft bath oil, Johnson's baby oil, Claubo, and mineral oil, which is common to the formulations of all of the above preparations. All of the materials, as well as deet, were quite effective. However, deet was observed to repel attacking midges while the preparations tested did not appear to act primarily as repellents, but physically trapped the midges and prevented bites due to their oiliness.

F. Lightweight net jackets treated with deet provided ca. 90% protection when field-tested in Panama against 5 species of biting midges, principally *C. furens* and *C. barbosai*.

G. Barrier treatment test results indicate that a 2 m high perimeter barrier of repellent-treated netting can provide relief from biting midge attacks for areas as large as 12 X 12 m for several days.

H. Studies were conducted to determine the effect of droplet size on insecticidal efficiency on biting midges. Results indicate that aerosols from standard ground ULV generators with a volume median diameter (VMD) of 8-10 μm should be highly efficient for biting midge control if applied in a manner that provides adequate exposure of the target insects.

I. Ground ULV tests against natural *C. mississippiensis* populations were conducted at Yankeetown with resmethrin. Very little control was noticed. The 2 major factors in achieving control appear to be the correct timing for the spray applications and the penetration of vegetative cover which, when dense, appears to restrict movement of the aerosol through the area.

J. Successful control was achieved with aerial applications of naled (Dibrom 14, 1 oz/acre, undiluted). Sprays at this dosage were highly effective (>90%) in controlling the natural population of biting midges on Parris Island.

K. Species composition, seasonal incidence, and relative abundance of adults were determined at Parris Island and Yankeetown.

L. Larval habitats of *Culicoides* at Parris Island and Yankeetown were characterized by plant cover. Seasonal fluctuations in larval populations were monitored.

M. Remote sensing techniques were evaluated to determine their usefulness in locating and quantifying larval habitats. These techniques seem to be very useful, and possibly could be used to quantify populations in a given geographical area.

N. In the laboratory, *C. mississippiensis* was successfully reared from egg to the adult stage. The optimum temperature for rearing was 20°C. Reared females were 100% autogenous and matured an average of 123 eggs. Blood-fed wild females with the same wing length as the reared females matured an average of 35 eggs.

O. Techniques were developed for blood-feeding field-collected *C. mississippiensis* on preserved bovine blood through a cloth-reinforced silicone membrane. There was no significant difference between the numbers of eggs matured by females which had fed on a human host (40±11) or through the membrane (36±13). This accomplishment and that of #N, along with an improved understanding of the mating behavior of *C. mississippiensis*, should eventually enable colonization of this species.

P. An effective portable DC interval suction trap was built and field-tested. Data obtained in this type of trap will assist us in determining the correct time and environmental conditions required to achieve the optimum level of control.

IV. Recommendations

A. These recommendations are made based on the data just summarized. Biting midge control is difficult, but three techniques that can be utilized immediately against the adult stage are: aerial applications with naled, personal protection (deet-treated net jackets and/or topical applications), and residual treatment of household screens.

1. Aerial application of naled at the rate of 1.0 oz/acre, applied with UC-123-K aircraft equipped with Tee Jet nozzles oriented 45° forward, from an altitude of 150-200 ft using 1000 ft swaths, is effective in reducing biting midge populations. Several applications made ca. 5-7 days apart should alleviate much of the problem. The actual number of

applications required will vary with the duration of the adult biting season, which will vary from location to location. An adult surveillance program should be established at each installation to obtain this information. Arrangements to treat military installations can be made with the Air Force's 355th TAS/Aerial Spray, Rickenbacker, ANGB, Ohio.

2. Personal protection can be obtained with deet-treated net jackets and topical applications of deet or several other commercially available products. The deet-treated net jackets are very useful for sentry and similar duties. They are not recommended for use where movement through heavily forested or jungle areas is required because they may become entangled in bushes and trees. These jackets are presently stocked in the U.S. Navy, Marine Corps and Air Force supply systems and are also available in the retail market for the general public. Personal protection can also be obtained with topical applications of deet, but concentrations of at least 25% active ingredient are required. Considerable protection can be obtained by using several commercially available products: Avon's Skin-So-Soft, Johnson's Baby Oil, Claubo, and mineral oil. Protection is achieved primarily because of the oily coating, which traps the midges on the skin surface, rather than causing complete repellency. These products were applied at full strength. One of these materials, Skin-So-Soft, is marketed as a bath oil to be used at the rate of 1/2 capful in a bathtub of water; since it is not labelled for application at full strength, further investigation may be warranted to determine the effect of repeated long-term skin applications.

3. Residual treatment of window screens and other screened areas will provide relief from adult midges which may enter buildings. Propoxur can be applied at 4.5% with a paint brush to outside surfaces of these screened areas. Retreatment of the screens is required every 2-3 weeks.

B. The following areas of study are warranted:

1. Further experimentation to fine-tune the aerial application techniques developed. Specifically, we feel that the costs of application may be reduced by formulation change and/or dosage reduction. Thus, we suggest that tests be conducted with naled at 0.75 oz/acre and that aqueous formulations be assessed.

2. Additional studies to better define the optimum timing of aerial applications to hit seasonal peaks and assess the long-range impact on both adult and immature population dynamics over a period of several years.

C. This contract has provided an opportunity for a sustained research effort to derive data necessary for recommendations on current insecticidal control and personal protection technology. Some of these measures recommended above provide only temporary relief from biting midges that may continually infiltrate from the breeding areas. Furthermore, resistance could develop to the insecticides evaluated during this contract if they were used in a manner which fosters the selection of tolerant individuals. Therefore, we recommend continuation, either in-house or by contract, of insecticide screening and development of improved delivery systems.

D. A biting midge pest management program should continue to be the primary long-range goal of research endeavors with biting midges, as the temporary measures currently available will not by themselves completely alleviate all the problems faced by the military. To accomplish this, we feel that the following areas need to be investigated:

1. Colonization of at least 1 species of salt marsh biting midge. This accomplishment will allow biologists to gain more insight into life table type data through studies on biotic potential, mating and feeding behavior, and diapause and estivation mechanisms. The data acquired will enhance the prospects of accurate simulation modelling for biting midge populations and computer-assisted assessment of prospective population management strategies. The existence of a self-sustaining colony will provide the test insects for laboratory evaluation of pheromones, insect growth regulators, conventional larvicides and adulticides, and parasites, predators and pathogens.

2. Conduct field studies on the swarming behavior, flight and dispersal characteristics and preferred resting sites of coastal biting midges. An increased knowledge of these behavioral characteristics can provide significant new perspectives that could lead to more effective population management strategies.

3. Conduct studies on attractancy of coastal biting midges, including natural and artificial attractants and pheromones. These studies will provide information that could lead to improved monitoring and surveillance systems and also to control strategies that incorporate attractant devices and methodology.

V. PUBLICATIONS PREPARED UNDER THIS CONTRACT

A. Published Manuscripts

Davis, E. L. 1981. Laboratory studies on life cycle development and adult blood-feeding of *Culicoides mississippiensis* (Diptera: Ceratopogonidae). M.S. Thesis, University of Florida. 107 pp.

Davis, E. L., J. F. Butler, R. H. Roberts, J. F. Reinert, and D. L. Kline. 1983. Laboratory blood-feeding of *Culicoides mississippiensis* Hoffman through a reinforced silicone membrane. J. Med. Entomol. 20: 177-182.

Davis, E. L., D. L. Kline, J. F. Reinert, R. H. Roberts, and J. F. Butler. 1983. The development of immature *Culicoides mississippiensis* (Diptera: Ceratopogonidae) under laboratory conditions. Ann. Entomol. Soc. Amer. 76: 918-924.

Haile, D. G., D. L. Kline, J. F. Reinert, and T. L. Biery. 1984. Effects of aerial applications of naled on *Culicoides* biting midges, mosquitoes and tabanids on Parris Island, SC. Mosq. News (in press).

Harlan, H. J., C. E. Schreck, and D. L. Kline. 1983. Insect repellent jacket tests against biting midges (Diptera: *Culicoides*) in Panama. Am. J. Trop. Med. Hyg. 32: 185-188.

Kline, D. L. 1981. Current status of *Culicoides* research at the USDA laboratory in Gainesville, Florida. Proc. Fla. Anti-Mosq. Assoc. 51: 71-75.

Kline, D. L. and R. H. Roberts. 1981. Effectiveness of chlorpyrifos, fenthion, malathion, and propoxur as screen treatments for control of *Culicoides mississippiensis*. J. Econ. Entomol. 74: 331-333.

Kline, D. L. and R. H. Roberts. 1982. Daily and seasonal abundance of *Culicoides* spp. biting midges (Diptera: Ceratopogonidae) in selected mangrove areas in Lee County, Florida. Fla. Entomologist 65: 126-135.

Kline, D. L., D. G. Haile, and K. F. Baldwin. 1981. Wind tunnel tests with seven insecticides against adult *Culicoides mississippiensis* Hoffman. Mosq. News 41: 745-747.

Kline, D. A., R. H. Roberts, and D. A. Focks. 1981. Extraction of larvae of the ceratopogonid biting midge, *Culicoides mississippiensis*, from salt marsh soils with a new agar technique. Mosq. News 41: 94-98.

Lacey, L. A. and D. L. Kline. 1983. Laboratory bioassay of *Bacillus thuringiensis* (H-14) against *Culicoides* spp. and *Leptoconops* spp. (Ceratopogonidae). Mosq. News 43: 502-503.

Roberts, R. H. and D. L. Kline. 1980. A method to evaluate insecticides as household screen treatments against *Culicoides* spp. biting midges. Mosq. News 40: 399-402.

Roberts, R. H. and D. L. Kline. 1981. Efficacy of insecticide-treated screens against biting midges (Diptera: Ceratopogonidae), *Culicoides* spp. Proc. Fla. Anti-Mosq. Assoc. 51: 41-43.

Schreck, C. E. and D. L. Kline. 1981. Repellency determinations of four commercial products against six species of ceratopogonid biting midges. Mosq. News 41: 7-12.

Schreck, C. E. and D. L. Kline. 1983. Area protection by use of repellent treated netting against biting midges (Diptera: Ceratopogonidae). Mosq. News 43: 338-342.

Schreck, C. E., D. L. Kline and N. Smith. 1979a. Protection afforded by the insect repellent jacket against six species of ceratopogonid biting midges. Mosq. News 41: 7-10.

Schreck, C. E., N. Smith and T. P. McGovern. 1979b. Repellency of selected compounds against two species of biting midges (Diptera: Ceratopogonidae: *Culicoides*). J. Med. Entomol. 16: 524-527.

B. Manuscripts Under Review

Kline, D. L., J. F. Kelly, and E. A. Ellis. A *Nosema* type of microsporidian infection in larvae of *Culicoides* spp. from salt marshes in Florida (submitted to J. Invertebr. Pathol.).

Kline, D. L., J. R. Wood, R. H. Roberts, and K. F. Baldwin. Laboratory effectiveness of four organophosphate insecticides against field collected salt marsh *Culicoides* spp. larvae (undergoing in-house peer review prior to submission to Mosq. News).

Wilkening, A. J., D. L. Kline, and W. W. Wirth. An annotated checklist of the Ceratopogonidae (Diptera) of Florida with a new synonymy (submitted to Fla. Entomologist).

C. Manuscripts in Preparation

Kline, D. L. and J. R. Wood. Habitat characterization of salt marsh *Culicoides* spp. larvae at Yankeetown, Florida: I. Marsh description and determination of types of breeding sites available (intended for Environ. Entomol.)

Kline, D. L. Habitat characterization of salt marsh *Culicoides* spp. larvae at Yankeetown, Florida: II. Fluctuations in seasonal abundance in transitional salt marsh areas (intended for Environ. Entomol.).

Wood, J. R. and D. L. Kline. Habitat characterization of salt marsh *Culicoides* spp. larvae at Yankeetown, Florida: III. Factors affecting seasonal and spatial distribution in salt marsh areas located adjacent to primary tidal creeks (intended for Environ. Entomol.).

Kline, D. L.. Seasonal abundance of adult *Culicoides* spp. in salt marsh areas at Yankeetown, FL (intended for J. Med. Entomol.).

Kline, D. L. and J. R. Wood. Habitat characterization of salt marsh *Culicoides* spp. larvae at Yankeetown, Florida: IV. Relative abundance of larvae in regularly and irregularly flooded salt marsh areas during different tidal cycles (intended for Bull. Entomol. Res.).

Kline, D. L., S. Ingle, J. R. Wood, R. Davis, and W. G. Hart. Habitat characterization of salt marsh *Culicoides* spp. larvae at Yankeetown, Florida: V. The reliability of remote sensing using aircraft to locate potential breeding sites (intended for Limnol. α Oceanogr.).

Kline, D. L. and A. J. Wilkening. The influence of environmental factors on the diurnal flight activity of salt marsh *Culicoides* spp. at Yankeetown, FL (intended for Bull. Entomol. Res.).

Kline, D. L., W. R. Dixon, and A. J. Wilkening. Description of a modified Koch-type AC-powered interval suction trap used to study flight activity of *Culicoides* spp. (intended for J. Med. Entomol.).

Kline, D. L., D. G. Haile, and J. F. Reinert. Evaluation of population surveillance methods for adult *Culicoides* spp. on Parris Island, SC (intended for Mosq. News).

Kline, D. L., D. G. Haile, F. Wilson and N. W. Pierce. Description of a DC-powered portable interval suction trap used to study flight activity of *Culicoides* spp. (intended for Entomol. Exp. Applic.).

Kline, D. L. and L. Wilson. Habitat characterization of salt marsh *Culicoides* spp. larvae at Parris Island, SC. I. Distribution and types of immature habitats (intended for Environ. Entomol.).

Kline, D. L. and L. Wilson. Habitat characterization of salt marsh *Culicoides* spp. at Parris Island, SC. II. Seasonal fluctuation in immature abundance at selected breeding sites (intended for Environ. Entomol.).

Kline, D. L. and L. Wilson. Species composition, seasonal and relative abundance of adult *Culicoides* spp. and mosquitoes in salt marsh areas at Parris Island, SC (intended for J. Med. Entomol.).

Kline, D. L. and L. Wilson. The influence of environmental factors on the diurnal flight activity of salt marsh *Culicoides* spp. at Parris Island, SC (intended for J. Med. Entomol.).

VI. References Cited

Dukes, J. C. and R. C. Axtell. 1976. Residual effectiveness of insecticide-treated screens for control of biting midges, *Culicoides furens* (Poey) (Diptera: Ceratopogonidae). Mosq. News 36: 488-491.

Haile, D. G., D. L. Kline, J. F. Reinert, and T. L. Biery. 1984. Effects of aerial applications of naled on *Culicoides* biting midges, mosquitoes and tabanids on Parris Island, SC. Mosq. News (in press).

Harlan, H. J., C. E. Schreck, and D. L. Kline. 1983. Insect repellent jacket tests against biting midges (Diptera: *Culicoides*) in Panama. Am. J. Trop. Med. Hyg. 32: 185-188.

Jamnback, H. 1961. The effectiveness of chemically treated screens in killing annoying punkies, *Culicoides obsoletus*. J. Econ. Entomol. 54: 578-580.

Jamnback, H. 1963. Further observations of the effectiveness of chemically treated screens in killing biting midges, *Culicoides sanguisuga* (Diptera: Ceratopogonidae). J. Econ. Entomol. 56: 719-720.

Kline, D. L. and R. H. Roberts. 1981. Effectiveness of chlorpyrifos, fenthion, malathion, and propoxur as screen treatments for control of *Culicoides mississippiensis*. J. Econ. Entomol. 74: 331-333.

Kline, D. L., D. G. Haile, and K. F. Baldwin. 1981. Wind tunnel tests with seven insecticides against adult *Culicoides mississippiensis* Hoffman. Mosq. News 41: 745-747.

Kline, D. L., R. H. Roberts, and D. A. Focks. 1981. Extraction of larvae of the ceratopogonid biting midge, *Culicoides mississippiensis*, from salt marsh soil with a new agar technique. Mosq. News 41: 94-98.

Koch, H. G., R. C. Axtell, and G. R. Baughman. 1977. A suction trap used for hourly sampling of coastal biting flies. Mosq. News 37: 674-680.

Linley, J. R., A. L. Hoch, and F. P. Pinheiro. 1983. Biting midges (Diptera: Ceratopogonidae) and human health. J. Med. Entomol. 20: 347-364.

Mount, G. A., N. W. Pierce, and K. F. Baldwin. 1975. Droplet size of aerosols dispersed by portable and truck-mounted generators. Mosq. News 35: 195-198.

Mount, G. A., N. W. Pierce, and K. F. Baldwin. 1976. A new wind-tunnel system for testing insecticidal aerosols against mosquitoes and flies. Mosq. News 36: 127-131.

Roberts, R. H. and D. L. Kline. 1980. A method to evaluate insecticides as household screen treatments against *Culicoides* spp. biting midges. Mosq. News 40: 399-402.

Roberts, R. H. and D. L. Kline. 1981. Efficacy of insecticide-treated screens against biting midges (Diptera: Ceratopogonidae), *Culicoides* spp. Proc. Fla. Anti-Mosq. Assoc. 51: 41-43.

Schreck, C. E. and D. L. Kline. 1983. Area protection by use of repellent treated netting against *Culicoides* biting midges. Mosq. News 43: 338-342.

Schreck, C. E., D. L. Kline and N. Smith. 1979a. Protection afforded by the insect repellent jacket against six species of ceratopogonid biting midges. Mosq. News 41: 7-10.

Schreck, C. E., N. Smith, and T. P. McGovern. 1979. Repellency of selected compounds against two species of biting midges (Diptera: Ceratopogonidae: *Culicoides*). Mosq. News 39: 739-742.